Symposium: Myosin Binding Protein-C: A Modulator of Cardiac Contractility

3038-Symp
Modulating Contraction by Binding of MyBP-C to Actin
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Myosin-binding protein C (MyBP-C) is a ~130 kDa protein of the thick filaments of vertebrate skeletal and cardiac muscle. It consists of a linear array of ten or eleven globular, 10-kDa domains from the immunoglobulin (Ig) and fibronectin type III families, and an additional, MyBP-C-specific motif. The cardiac isoform, cMyBP-C, plays a key role in modulating cardiac function, and mutations in MyBP-C cause heart disease. Despite its discovery 40 years ago, the mechanism of MyBP-C function remains poorly understood. *In vitro* studies suggest that it could modulate contraction by binding to thin filaments, but there has been no evidence for this *in situ*. We used electron tomography of exceptionally well-preserved skeletal muscle to study the 3D organization of MyBP-C in the intact sarcomere. The tomogram shows that MyBP-C projects perpendicular to the thick filament surface and reaches neighboring thin filaments. This thick-thin filament bridge suggests a possible physical basis for modulating filament sliding and thus contraction. *In vitro*, binding to actin has been shown to occur via MyBP-C's N-terminal end. To understand the structural basis of this binding, we used negative stain electron microscopy and 3D reconstruction to study F-actin decorated with bacterially expressed N-terminal cMyBP-C fragments. Clear decoration was obtained under a variety of salt conditions. 3D reconstructions showed MyBP-C density starting over subdomain 1 of actin and extending tangentially towards actin's pointed end. Molecular fitting with an atomic structure of a MyBP-C Ig domain suggested that most of the N-terminal domains may be well ordered on actin. The location of binding was such that it appeared to overlap the relaxed (low Ca^{2+}) position of tropomyosin but not the activated position. This suggests that MyBP-C might help determine the state of thin filament activity by modulating tropomyosin position on actin.

3039-Symp
Dynamic Regulation of Contraction by Cardiac Myosin Binding Protein-C
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Cardiac myosin binding protein-C (cMyBP-C) is a thick filament associated protein that performs both regulatory and structural roles within cardiac sarcomeres. It is a member of the immunoglobulin (Ig) superfamily of proteins consisting of 8 Ig- and 3 fibronectin (FNIII)-like subdomains along with a unique regulatory sequence referred to as the M-domain whose structure is unknown. Here we used atomic force microscopy (AFM) to probe the structure and mechanical properties of the different subdomains of native and recombinantly expressed cMyBP-C molecules. Results demonstrate that cMyBP-C exhibits complex mechanical behavior under load and contains multiple domains with distinct mechanical properties. The Ig and FNIII-like domains unfold over a range of relatively low forces (50-190 pN), whereas the M-domain is readily extensible at forces $<$50 pN and is likely to be an intrinsically disordered segment of cMyBP-C. Additional extensible segments of cMyBP-C are likely to include linkers between the Ig domains such as the proline-alanine rich sequence between domains C0 and C1. Taken together these results suggest that cMyBP-C is compliant and readily extensible, potentially conferring structural and functional plasticity to cMyBP-C during the course of a single heart beat. Supported by NIH HL080367.

3040-Symp
Cardiac Myosin Binding Protein-C Phosphorylation, Contractile Function and Cardioprotection
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Cardiac myosin binding protein-C (cMyBP-C) is a sarcomeric thick filament assembly protein with regulatory functions in the heart. The cMyBP-C protein differs from the skeletal isoform in that it has a small insertion near the carboxyl terminus that contains 3 phosphorylatable serines, Ser-273, −282 and −302. While the precise functional correlates of cMyBP-C phosphorylation remain obscure, we do know that cMyBP-C is targeted by multiple kinases, such as PKA, PKC, RSK, PKD, CaMKII and PKG, suggesting that it plays a vital role in cardiac signaling. We previously reported that cMyBP-C phosphorylation is essential for normal heart function and that Ser-282 phosphorylation is critical for the subsequent phosphorylation of Ser-302 and normal cardiac function. However, the role of Ser-282 cMyBP-C phosphorylation in cardiac function, particularly as it affects contractile properties and sarcomere organization, is unclear. Therefore, to better understand the mechanisms and significance of cMyBP-C phosphorylation, we established several transgenic mouse models to determine the necessity and sufficiency of Ser-282 phosphorylation for normal cardiac function. Our findings suggested that cMyBP-C phosphorylation at Ser-282 is essential for normal cardiac function and that dephosphorylation at this site accelerates cMyBP-C degradation and cleavage of a 40 kDa fragment. During MI, we showed that cMyBP-C is extensively fragmented when dephosphorylated and that such fragmentation correlates well with contractile dysfunction and heart failure. Meanwhile, we also established that the release of cMyBP-C in the blood post-MI could be a potential diagnostic biomarker for MI. Overall, these studies show that Ser-282 phosphorylation is a critical determinant of Ser-302 phosphorylation and that cMyBP-C dephosphorylation accelerates its degradation and release into the circulation. In conclusion, we provide strong evidence that cMyBP-C phosphorylation directly affects the heart's contractile properties, sarcomere organization and cardioprotection.

3041-Symp
Cardiac Myosin Binding Protein C Phosphorylation in Human Cardiac Disease
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During recent years it has become increasingly evident that cardiac myosin binding protein C (cMyBP-C) exerts an important role in regulation of sarcomere function with consequences for *in vivo* cardiac performance. The functional role of cMyBP-C is tightly regulated by kinase-mediated phosphorylation. The most important kinase which phosphorylates cMyBP-C *in vivo* is protein kinase A (PKA), which is activated upon stimulation of the β-adrenergic receptors during exercise. In end-stage failing human myocardium, reduced phosphorylation of cMyBP-C has been reported using 1-dimensional and 2-dimensional gel electrophoresis. This reduced phosphorylation has been attributed to down-regulation and desensitization of the β-adrenergic receptor pathway in end-stage human heart failure. Low levels of cMyBP-C phosphorylation were also found in patients with familial hypertrophic cardiomyopathy (FHC), which is frequently caused by mutations in genes encoding sarcomeric proteins, with one exception: in FHC patients with mutations in the gene which encodes cMyBP-C (*MYBPC3*), phosphorylation of cMyBP-C was unaltered compared to non-failing donor hearts. cMyBP-C can be phosphorylated *in vivo* on at least three serine sites (Ser273, Ser282 and Ser 302), all of which are located in the cardiac isoform specific M region. At least one other site should exist in humans. With tandem mass spectrometry we recently identified a fourth phosphorylation site on Ser133, which is present in the Pro-Ala rich region that links the C0 and C1 domains. Preliminary data indicate that Ser133 is not phosphorylated by PKA. Analysis of phosphorylation on Ser133 in failing and donor samples revealed lower levels of Ser133 phosphorylation in several forms of cardiac disease compared to non-failing myocardium. Overall, our studies indicate that diverse cMyBP-C phosphorylation patterns exist in human cardiomyopathies, which may in part underlie sarcomere dysfunction observed in human heart failure.

Symposium: Materials Science Meets Biology

3042-Symp
Engineering Cooperative Nanosytems
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Our laboratory is interested in engineering tools and systems using nanoparticle scaffolds to transform the diagnosis and treatment of cancer. We aim to integrate nanomaterials with enhanced nanoscale properties and bioresponsive functionalities with our knowledge of the tumor microenvironment to explore this paradigm. Towards this end, we have developed and investigated nanoparticle conjugates based on three nanoparticle cores that harness features of the nanoscale: semiconductor quantum dots that exhibit size-based optical properties, dextran-coated iron oxide particles whose assembly alters the spin-spin relaxation time of hydrogen protons on magnetic resonance imaging, and polymer-coated gold nanorods that interact resonantly with near-infrared light. Our studies have shown how these nanoparticles specifically designed to